

transgene (FIG. 1B). Results from one experiment representative of two independent evaluations are shown. Expression of the GFP transgene after transduction of human CD34⁺ cells or HeLa cells. Results are represented as histograms of GFP fluorescence intensity (x-axis, four-log scale) versus cell number (y-axis, linear). GFP⁺ cells (within marker) were analyzed for percentage (lower number) and median of fluorescence intensity (upper number). Percentages were omitted in HeLa cells since the histograms were obtained from titration experiments of the corresponding vectors (FIG. 1A). Presence of the GFP transgene after transduction and expansion of human CD34⁺ cells. Cellular extracts equivalent to 5000 cells (upper panel) were amplified as described in the section entitled Examples with GFP-specific primers, together with IL-2 specific primers as internal controls. Sizes of the corresponding PCR products are indicated. Lanes are: M, molecular weight marker; HeLa (negative control for GFP, positive control for IL-2); 4.5, a clone of HeLa containing one copy of HIV-CMV-GFP vector; 0, untransduced CD34⁺ cells; MLV-CMV-PGK-EF1, CD34⁺ cells transduced with the corresponding vectors (see FIG. 2A); CMV-EPO, CD34⁺ cells transduced with HIV-CMV vector (lane CMV) and analyzed after expansion and differentiation into erythroid cells (see text); CMV-GM, same as CMV-EPO but analyzed after expansion and differentiation into monocytic cells (see text); PGK-EPO and PGK-GM, same as CMV-EPO and CMV-GM but from CD34⁺ cells transduced with HIV-CMV vector (lane PGK). To ensure for proportionality, cellular extracts equivalent to 1700 cells (lower panel) were amplified separately (FIG. 1B).

FIG. 2. Effect of MOI on transduction efficiency of lentivectors in human CD34⁺ cells. 10⁵ CD34⁺ cells were transduced with various doses (1, 2, 5, 10, 20 and 50x10⁵ TU) of EF1α-GFP lentiviral vector allowing multiplicities of infection (MOI) ranging from 1 to 50. GFP expression was analyzed by flow cytometry after 4 days. Upper panel: Data from one representative experiment are shown as frequency histograms of GFP fluorescence intensity versus cell number (events). Gates for GFP⁺ cells were setup according to untransfected cells (MOI 0). Lower panel: Mean percentages of GFP⁺ cells obtained with MOIs of 0 to 10 and 10 to 50 are represented in the main panel and in the